



## RESEARCH PAPER

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## The effect of fermented organic feed usage on the health status of kampong chicken

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**Key words:** Organic feed, Corn, Ricebran, *Saccharomyces cerevisiae*.

<http://dx.doi.org/10.12692/ijb/12.4.35-42>

Article published on April 08, 2018

### Abstract

This research aims to determine the effect of the use of fermented organic feed to the health status of Kampong chickens based on the number of bacteria in the intestine and their blood profile. The experiment method with Completely Randomized Design (CRD) was utilized in this study. There were four treatments, To = organic feed without fermentation, T1 = organic feed with fermented rice bran, T2 = organic feed with fermented corn and T3 = a combination of fermented rice bran and corn. The fermentation was done using yeast, *Saccharomyces cerevisiae*, each of these treatments consisted of ten chickens. The parameters measured were the colony number of *Escherichia coli*, *Salmonella sp.* in the intestine, the number of erythrocytes, leukocyte, level of hemoglobin and hematocrit, atotal of plasma protein and blood glucose. The data were analyzed by quantitative descriptive analysis. The results show that the fermented rice bran provides the best growth inhibitory effect of *E. coli*, at  $232.6 \times 10^3$  cfu/ml, towards *Salmonella sp.* has been reached by the combination of fermented rice bran and corn, at  $794 \times 10^3$  cfu/ml. The number of erythrocytes, leukocytes, hematocrit level and a total of plasma protein of blood samples obtained good results by the standard of the literature. The hemoglobin level in rice bran and corn fermented according to the standard level, although in control and the combination of fermented rice bran and corn were less than standard, 6.82 g/dl and 6.30 g/dl respectively.

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## Introduction

Some disadvantages caused by salmonellosis are disruption of growth and production, an increase in the number of culled chicken and an increase of the chicken susceptibility to other diseases. Salmonellosis disease has a significant role in public health, so products from poultry contaminated with *Salmonella* sp. must be destroyed, so the company livestock losses. *E.coli* is pathogenic, and its infection can be in the form of embryonic death in hatching eggs, infection of the yolk sac, *omphalitis*, colisepticemia, airsacculitis, enteritis, infection of reproductive organs (salpingitis). Colibacillosis also supports the emergence of complex diseases of the respiratory tract, gastrointestinal and reproduction, which are quite difficult to eradicate.

A new breakthrough is needed to make an organic feed for Kampong chicken to overcome the both of salmonellosis and colibacillosis disease in Indonesia. The availability of high quality of the organic feed needs to be achieved by the fermentation process involving microorganisms, like yeast, *S. cerevisiae* is the famous type of yeast, as probiotic in animal nutrition (Simon *et al.*, 2001). It leads to benefits for the host by improving its microbial balance (Fuller, 1989). Probiotics have been tested for their efficacy at controlling *Salmonella* colonization in the broiler, reduction in the colonization achieve 60% (Higgins *et al.*, 2007), 44% (Stern *et al.*, 2001).

The population of *E. coli* is significantly decreased by the addition of *S. cerevisiae* and Mannan Oligosaccharides (MOS) combination in the cecum ( $P < 0.01$ ) (Janardhana *et al.*, 2009). Other study reported that fermented rice bran using *S. cerevisiae* shown to increase levels of dietary fiber, from 28.20 % to 38.90 %, an increase insoluble fiber from 71.10 % to 78.60 and decreased the soluble fiber from 2.85 % to 2.22%. Insoluble fiber plays a role in inhibiting the attachment of pathogenic bacteria to the intestinal mucosa wall (Geetha *et al.*, 2015).

Fermented feed increases digested and absorbed nutrients in the way of in vivo. It will consequently

provide healthy blood profiles of the chicken. Due to the healthy blood profiles, the performance of Kampong chicken production can be maximum by their genetic potentials. From the above description, there needs to be further study on the effect of using the fermented organic feed on the health status of chicken, based on the number of colonies of pathogenic bacteria in the intestines and blood profiles. It is expected that fermented organic feed can inhibit the growth of pathogenic bacteria in the intestines and improvement of blood profile, which in turn the chicken can proliferate, be healthy and disease resistant, therefore the maximum productivity can be achieved.

## Materials and methods

### *Experimental design, chicken, and management.*

This study using 40-day old chick (DOC) are reared for 42 days, using the open house, the floor covered with a rice husk as a litter. Chicken randomly divided into four treatments group, the experimental methods are used the complete random design (CRD), each treatment consisting of ten chickens. During the trial, feed and drinking water provided in ad libitum, the treatments feed were consumed by chicken after 14 old.

The treatment in this research consisted of To = organic feed without fermentation, T1 = organic feed with 0,2% fermented rice bran, T2 = organic feed with 0,2% fermented corn and T3 = organic feed with 0,2% a combination of fermented rice bran and corn. The complete compositions of the feed are presented in Table 1.

### *Feedstuff fermentation*

The fermentation process was done by mixing corn or rice bran with a little bit of water, then steamed for 30 minutes after the water boiled. After that, let the feedstuff cooled down until the temperature reached 30°C - 40°C. Once the feedstuff reached the predetermined temperature, sown *S. cerevisiae* on the top of it and let them incubated in an airtight container for three days. Finally, mixed the

fermented feedstuff with others feedstuff which were not fermented by every treatment formulation.

#### *Isolation and identification of bacteria of feces samples*

Isolation and identification of bacteria with feces, like the samples, were diluted by using buffered peptone water solution with comparison 1:9. Then, done dilution in serial  $10^{-1}$  until  $10^{-3}$ , each dilution grew into media, called Eosin Methylen Blue Agar (EMBA) and Salmonella Shigella Agar (SSA), and was incubated at  $37^{\circ}\text{C}$  for 24 hours. After incubation, characteristic metallic-sheen colonies on EMBA and pale yellow colonies with black spots on their centers on SSA were considered to be possible *E. coli* and *Salmonella sp.*, respectively (Phokela *et al.*, 2011).

#### *The parameters measurement of blood samples*

At the end of the experiment, a blood sample was taken from a vein a chicken with needle sterilized and be accommodated on syringe sterile labeled that

contains Ethylene Diamine Tetraacetic Acid (EDTA) as an anticoagulant. The number of red blood cell (RBC), white blood cells (WBC), the concentration of hemoglobin (Hb), and packed cells of volume (PCV) in the sequence is measured using Neubauer hemocytometer, cyanomethaemoglobin, and microhematocrit (Coles, 1986). The analysis of the data used descriptive and interpretative analysis of quantitative method.

### **Results and discussion**

#### *The effect of the use of feed treatment towards intestinal pathogen bacteria of kampong chicken*

From the Table 2 above, it was shown that the use of organic feed with fermented rice bran provides the best growth inhibitory effect of *E. coli*, at  $232.6 \times 10^3 \text{ cfu/ml}$ . It is related to the increase in the number of colonies *S. cerevisiae* in the intestine because the fermented rice bran provides sufficient nutrients needed for the growth of *S. cerevisiae*.

**Table 1.** Feed Formulation of Kampong Chicken in Grower Period.

Feed Ingredients	Treatment			
	P0	P1	P2	P3
Corn	28	31	23	40
Rice bran	17	11	16	7
Oil	10.5	9.6	11.2	7.8
Soybean meal	37	39	17	33
Fish meal	5	4.5	-	-
MBM ( <i>Meat and Bone Meal</i> )	12	6	10.5	8.9
Limestone flour	1.3	1.3	1.3	1.4
Medical herbs	0.5	0.5	0.5	0.5
Diphosphate	0.5	0.5	0.5	0.5
Mineral	0.5	0.5	0.5	0.5
Total	100	100	100	100

The use of the fermented rice bran is proven to be able to increase the number of beneficial bacteria in the intestine that is the Lactic Acid Bacteria (LAB) and yeast, but it can decrease pathogenic bacteria, namely *E. coli*. (Koc *et al.*, 2010). The fermented rice bran contains indigestible oligosaccharide, resistant starch, and oligopeptides, which are the effective nutrient sources for the growth of intestinal probiotics

including *S. cerevisiae*. Organic feed with fermented rice bran was a suitable substrate for the growth of *S. cerevisiae* in the intestine so that the increase of extracellular enzymes, such as *amylases*, *cellulases*, *xylanases*, *esterases*, and *proteases*, as well as the results of its secretion, will occur. Besides, *S. cerevisiae* can also produce phenol and other compounds that are essential for health and act as

anti-mutagenic, anti-oxidants, and anti-microbes (Geetha *et al.*, 2015).

The results have shown that the lowest growth of the *Salmonella sp.* is reached on the use of organic feed with fermented rice bran and corn. It is related to the

rapid growth of *S. cerevisiae* that makes the content of MOS in *S. cerevisiae* also increase.

The elevation of the MOS is essential for the inhibition of *Salmonella sp.* attachment to the mucosa in the intestinal wall.

**Table 2.** The Effect of the Use of Feed Treatment towards Intestinal Pathogen Bacteria of Kampong Chicken (*Gallus domesticus*) ( $\times 10^3$ cfu/ml).

Pathogens bacteria in intestines	Treatment			
	To	T1	T2	T3
<i>Escherichia coli</i>	912 $\pm$ 154.76	232.6 $\pm$ 40.52	1361 $\pm$ 349.71	1000 $\pm$ 331.16
<i>Salmonella sp</i>	982 $\pm$ 136.20	877 $\pm$ 333.27	1626 $\pm$ 307.87	794 $\pm$ 187.87

It is in line with the state that the use of the MOS can kill five out of the seven strains of the *E. coli* and seven out of the ten strains of the *S. typhimurium* and *S. enteridis*. However, the use of the MOS cannot kill the strain of *S. pullorum*, *S. choleraesuis*, and *Campylobacter*. The results of a challenge test against *S. typhimurium*29E ( $10^4$ cfu) at the age of three-day-old chicks, with the MOS level given 4000 ppm showing a reduction in the colonies number of *S. typhimurium* from 5.40 up to 4.01 log cfu at the tenth day (Spring *et al.*, 2000).

highest growth of *E. coli* and *Salmonella sp.*, in which the growth of the *E. coli* is 1361  $\times 10^3$ cfu/ml and the growth of the *Salmonella sp.* is 1626  $\times 10^3$ cfu/ml. It was due to the dietary fiber content in the corn was much lower than the rice bran. Fermentation of the rice bran using *S. cerevisiae* is proven to increase the level of dietary fiber, from 28.20% up to 38.90%, insoluble fiber from 71.10% up to 78.60%, while the soluble fiber is proven to decrease from 2.85% up to 2.22%. The role of the insoluble fiber is to hamper the attachment of pathogenic bacteria on the intestinal wall mucosa (Geetha *et al.*, 2015).

Organic feed with fermented corn has led to the

**Table 3.** Blood profile of 42 day-old-Kampong chicken.

Blood Profile	Standard (Suryaniet <i>et al.</i> , 2014)	Treatments			
		P0	P1	P2	P3
Erythrocyte ( $\times 10^6/\mu\text{l}$ )	2.5-3.5	2.65 $\pm$ 0.15	2.65 $\pm$ 0.16	2.84 $\pm$ 0.22	3.03 $\pm$ 0.44
Leukocyte ( $\times 10^3/\mu\text{l}$ )	12 - 30	16.66 $\pm$ 6.06	16.46 $\pm$ 2.08	14.85 $\pm$ 2.29	15.28 $\pm$ 2.65
Hemoglobin (g/dl)	7 - 13	6.82 $\pm$ 0.97	7.86 $\pm$ 1.44	7.40 $\pm$ 1.27	6.30 $\pm$ 1.01
Hematocrit (%)	22 - 35	29.76 $\pm$ 3.74	34.14 $\pm$ 7.03	30.96 $\pm$ 2.67	29.08 $\pm$ 2.77
TPP level (g/dl)	4.5 - 5.5	10.01 $\pm$ 0.99	10.99 $\pm$ 0.83	10.97 $\pm$ 1.41	11.03 $\pm$ 1.17
Glucose level (mg/dl)		123.32 $\pm$ 8.21	137.93 $\pm$ 15.66	173.16 $\pm$ 9.92	181.02 $\pm$ 12.53

*The effect of the use of feed treatment towards the blood profiles of kampong chicken*

Erythropoiesis process that occurs in the bone marrow requires many nutrients, such as amino acids, folic acid, iron, vitamin B<sub>12</sub> absorbed from the small intestine. The number of erythrocytes in the control and treatment of organic feed is still within the normal range, between 2.65 to 3.03 million cells/ml. The data shows that all the chickens in the trial process are in the healthy condition in which the nutritional

needs are supplied. This result is consistent with the statement Silvia *et al.* (2008) that the addition of probiotic and synbiotic to broiler diet had no significant effects on RBC count at 42 days.

The role of yeast in the fermented organic feed significantly affects the number of erythrocytes. It is related to the increased metabolic activity by *S. cerevisiae*, which spurs an increase in the number of erythrocytes in the body of chickens with fermented organic feed. The reason is also consistent with Koc *et*

*al.* (2010) who state that use of *S. cerevisiae* affects the microbiota in the ileum that is an increasing number of LAB and yeast, as well as a decrease in the number of *E. coli*. Further, they also say that *S. cerevisiae* and MOS combination supplementation substantially increase the population of lactic acid bacteria and yeast in the cecum content. Besides, the removal of potential pathogens from the intestinal tract of growing animals may provide a more favorable environment for the digestion, absorption, and metabolism of growth-enhancing nutrients. Otherwise also that the effect in prebiotic-treated groups, MOS and fructooligosaccharides (FOS) which is a mayor gut-associated lymphoid tissue (GALT) were similar to those in the antibiotic-treated groups. The hypothesized that these prebiotic-mediated immunological changes might be in part due to direct interaction between prebiotic and gut immune cells as well as due to an indirect action of prebiotics via colonization of beneficial microbes and microbial products that interact with immune cells (Janardhana *et al.*, 2009).

The level of leucocytes of the experiment chicken is still in the normal range, between 14.850-16.660 cells/ $\mu$ l. The organic feed with fermented bran provides the highest number of leukocytes, equal to the controlled group. It shows an increase in phagocytic activity to destroy foreign substances, including bacteria. It is consistent with Suryani *et al.* (2014), that neutrophil is leucocytes cells that act as the body's first line of defense against bacteria, showing phagocytosis activity to attack bacteria. Neutrophils will appear in large numbers during inflammation. Organic feed with fermented corn showed the lowest number of leucocytes because levels of insoluble dietary fiber are shallow, so that decrease the inhibitory effect of microbial pathogens.

As stated by Koc *et al.* (2010), the highest weight of duodenum is found in broiler chickens with control feed, without the addition of *S. cerevisiae* or MOS or feed with MOS supplementation only. Duodenum weight of broiler chickens supplemented by *S. cerevisiae* with or without MOS is proven significant to reduce the weight of gizzard and duodenum,

because of the balance of microbial populations in the gastrointestinal tract, which is central to support the health and improvement of the performance of the chicken production. The gastrointestinal system will become unwieldy without the supplementation of *S. cerevisiae* on the feed.

MOS which is derived from the cell wall of *S. cerevisiae* has shown promising effects such as decreasing pathogenic microflora of the gut and stimulating a strong immune response. Also, balancing the intestinal environment and elevating the strength of the intestinal mucosa in studies with poultry (Iji *et al.*, 2001; Spring *et al.*, 2000; Hooge, 2004).

The increase of the hemoglobin concentration should be positively correlated with the increase of the number of erythrocytes. That is because Hemoglobin is the pigment of the erythrocytes consisting of a complex protein called globin and heme-containing iron. Hemoglobin is the most dominant protein in the blood, about 10% of total blood and 90% of the dry weight of erythrocytes (Wijastuti *et al.*, 2013). The results of this research have shown that the highest hemoglobin concentrations achieved in chickens fed with the organic feed of fermented rice bran. However, the highest number of erythrocytes is achieved in chickens fed with the organic feed of fermented rice bran and corn. This discrepancy could be explained by the statement that erythrocytes play an essential role in transporting O<sub>2</sub> and CO<sub>2</sub>. The surface shape and size of erythrocytes are the crucial factors for the exchange of gases in the network. Small erythrocytes provide the faster speed of gas exchange than the big erythrocytes. Similarly, elliptical erythrocytes cell is more efficient in gas exchange than spherical erythrocytes, with the same amount or volume. The longest size of erythrocytes is achieved in broiler chickens fed with feed supplemented by *S. cerevisiae*, while the broadest size of the erythrocytes is achieved in broiler chickens fed with feed supplemented by MOS (Koc *et al.*, 2010).

The higher hemoglobin concentration in the chicks received probiotics, and synbiotic may be due to the acidic media of the alimentary tract caused by prebiotic fermentation which resulted in better iron salt absorption from the small intestine. It may also cause better vitamins B-complex production by beneficial bacteria which may result in positively affecting blood-forming processes (Kander, 2004).

Hematocrit is a percentage (%) of the content of cells in the blood. Hematocrit value is the ratio between the numbers of cells and blood plasma. Hematocrit level depends violently on the number of erythrocytes because erythrocytes are the most abundant mass of cell in the blood. All of the experiment chickens show hematocrit value in the standard level, around 29.08% to 34.14%. Thus, all the chicken show a healthy condition. This normal hematocrit value is caused by the number of erythrocytes in all experiment chickens that show the normal amount. The highest hematocrit value which is achieved in chickens fed with organic feed with fermented rice bran is about 34.14%.

The highest level of plasma protein is achieved by the chickens fed with the organic feed of fermented bran and corn. The fermentation done on bran and corn has led to an increase of protein in feed due to the enzymes secretion from *S. cerevisiae*, including phytase enzyme that describes the bond between phytic acid and protein. It is in line with the state that the fermentation of rice bran after heating to 30°C for three days leads to an increase of the total protein from 17.60% to 18.40%. It is also stated that the fermentation of *S. cerevisiae* increases the protein digested by the way of in vitro from 3.7% up to 6.37%. The increase of protein in fermented rice bran is due to the decrease of the level of phytic acid anti-nutrition from 5.107% before the fermentation into 4.819% after the fermentation (Geetha *et al.*, 2015). The lowest content of blood glucose is achieved by chicken fed with organic feed without fermentation, and the highest content of blood glucose is achieved by the chicken fed with the organic feed with fermentation of bran and corn.

There is a higher tendency of blood glucose level in the organic feed of fermented corn than the fermented rice bran. That is because the percentage of the corn used in the feed is higher than the percentage of the rice bran. The fermented rice bran is about 17% of the total feed, while the fermented corn is about 28% of the total feed. Also, the content of energy in the corn is higher than that of the rice bran. The content of the energy in the yellow corn is about 3370 kcal/kg, while in the rice bran the content of the energy is 2860 kcal/kg (Asiyah *et al.*, 2013). The content of starch in the corn which is higher than that of the rice bran is converted into the glucose by the amylase enzyme of the yeast containing *S. cerevisiae* so that the blood glucose level of the fermented corn is higher than the fermented rice bran. The role of the enzyme to convert a substrate into a product is determined by the balance between the concentration of the substrate and the enzyme. The concentration balance of feed starch as the substrate with the enzyme of amylase from the best *S. cerevisiae* is on the organic feed with the fermented rice bran and corn to achieve the highest glucose level.

## Conclusion

Based on the results of the research, it can be concluded that the organic feed with fermented rice bran gave the best effect on profile blood and inhibition *E. coli* in the intestines. Organic feed with a combination fermentation rice bran and corn gave the effect of highest on inhibition *Salmonella sp.* in the intestines.

## Acknowledgement

Gratitude is devoted to Ministry of Research and Technology Directorate General of Higher Education for funding this research. Gratitude is also directed to Directorate of Research and the Community Service University of Muhammadiyah Malang for the opportunity to carry out this research.

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